HETEROGENEITY OF CRYSTALLINE BETA-LACTOGLOBULIN

Sir:

That crystalline β-lactoglobulin is not a homogeneous protein was indicated by the solubility measurements of Grönwall[1] and by the electrophoretic results of Li.[2] Our experiments with this protein confirm these reports and indicate a relationship between the solubility of the protein and its composition as defined by the electrophoretic patterns obtained at *p*H 4.7.

β-Lactoglobulin was prepared by the method of Palmer[3] which involves the fractionation of milk whey with ammonium sulfate after the removal of casein with acid. The isolated β-lactoglobulin was recrystallized four times by dialyzing away the salt from sodium chloride solutions at the isoelectric point. Electrophoretic studies on a 1% solution of this material in acetate buffer of ionic strength 0.1, *p*H 4.8, showed the presence of a two component system. The same preparation was electrophoretically homogeneous on the alkaline side of the isoelectric point.

Fractionation experiments on the whey proteins from the filtrate after the partial removal of crystalline β-lactoglobulin gave crystalline fractions with a mobility comparable to that of the usual β-lactoglobulin at *p*H 8.3. Electrophoresis *p*H 4.8, however, indicated a variation in the concentration of the components in each of the fractions. These new crystalline fractions also varied from the standard preparation in that they showed a greater solubility in water and in dilute salt solutions.

Crystallization of the standard preparation by dialysis from an acetate buffer solution, varied with respect to *p*H, gave fractions with a partial separation of the components as indicated electrophoretically. Partial separation of β-lactoglobulin has also been obtained by fractionation with alcohol at low temperatures. The fractions obtained by alcohol have properties in agreement with the data reported in the table below for preparations obtained by the other methods. These results will be reported subsequently.

The solubility experiments were made as described by Grönwall.[1] In every case a suspension of the crystalline material containing 7.6 ± 0.1 mg. protein nitrogen per ml. was equilibrated for a twenty-four hour period. The protein nitrogen was then determined on the supernatant liquid after centrifugation. The table illustrates the variations in the electrophoretic components of a number of preparations at *p*H 4.8 and the solubility of these crystalline fractions at *p*H 5.2 ± 0.1.

Preparation	% Composition, $\mu \times 10^5$ 1.4–1.5	2.2–3.2	Solubility (mg. N/cc.) at 25° in H_2O	in 0.02 *M* NaCl
B[a]	28	72	0.08	1.1
A[a]	38	62	.10	1.3
Standard	40	60	.12	1.5
K[b]	46	54	.18	1.7
L[b]	54	46	.13	1.6
M[b]	59	41	.19	1.9

[a] Preparations made from the standard preparation by means of acetate buffer of varying *p*H. [b] From filtrate of standard β-lactoglobulin preparation.

The variation in solubility in salt solutions of preparations with different electrophoretic compositions is an adequate explanation for the divergent solubility data reported by Palmer[3] and Grönwall.[1]